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SANTA CRUZ BIOTECHNOLOGY, INC.

TC 21 siRNA (m): sc-41862

BACKGROUND

H-, K- and N-Ras represent the prototype members of a family of small G proteins that are frequently activated to an oncogenic state in a wide variety of human tumors. Activation is due to point mutations at either position 12 or 61 within their coding sequence. Such mutations cause these proteins to be constitutively converted to their active GTP-bound, rather than the inactive GDP-bound state. The related human R-Ras gene was initially cloned by low stringency hybridization methods. Position 38 and 87 (analogous to position 12 and 61 in H-Ras) mutants of R-Ras have been shown to be capable of activating oncogenic function. An additional member of the Ras oncogene family, designated TC 21 (or R-Ras-2) is most closely related to R-Ras. While wild type TC 21 does not exhibit transforming potential *in vitro,* mutant forms of TC 21 that possess amino acid substitutions analogous to those that activate Ras oncogenic potential, exhibit potent transforming activities comparable to the activity characteristic of the known oncogenic Ras proteins.

REFERENCES

1. Lowe, D.G., et al. 1987. Structure of the human and murine R-Ras genes, novel genes closely related to Ras proto-oncogenes. Cell 48: 137-146.
2. Lowe, D.G., et al. 1987. Heterologous expression and characterization of the human R-Ras gene product. Mol. Cell. Biol. 7: 2845-2856.
3. Barbacid, M. 1987. Ras genes. Ann. Rev. Biochem. 56: 779-827.
4. Bos, J.L. 1989. Ras oncogenes in human cancer: a review. Cancer Res. 49: 4682-4689.
5. Drivas, G.T., et al. 1990. Characterization of four novel Ras-like genes expressed in a human teratocarcinoma cell line. Mol. Cell. Biol. 10: 1793-1798.
6. Saez, R., et al. 1994. Oncogenic activation of human R-Ras by point mutations analogous to that of prototype H-Ras oncogenes. Oncogene 9: 2977-2982.
7. Cox, A.D., et al. 1994. R-Ras induces malignant, but not morphologic, transformation of NIH/3T3 cells. Oncogene 9: 3281-3288.
8. Graham, S.M., et al. 1994. Aberrant function of the Ras-related protein TC 21/T-Ras2 triggers malignant transformation. Mol. Cell. Biol. 14: 4108-4115.

CHROMOSOMAL LOCATION

Genetic locus: Rras2 (mouse) mapping to 7 F1.

PRODUCT

TC 21 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TC 21 shRNA Plasmid (m): sc-41862-SH and TC 21 shRNA (m) Lentiviral Particles: sc-41862-V as alternate gene silencing products.

For independent verification of TC 21 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41862A, sc-41862B and sc-41862C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TC 21 siRNA (m) is recommended for the inhibition of TC 21 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

TC 21 (F-8): sc-166262 is recommended as a control antibody for monitoring of TC 21 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TC 21 gene expression knockdown using RT-PCR Primer: TC 21 (m)-PR: sc-41862-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.